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## **Collectrin and ACE2 in renal and intestinal amino acid transport**

Singer, D ; Camargo, S M R

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**Tmem27 and ACE2 in renal and intestinal amino acid transport**

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**Abstract:**

Neutral amino acid transporters of the SLC6 family are expressed at the apical membrane of kidney and/or small intestine, where they (re-)absorb amino acids into the body.

In this review we present the results concerning the dependence of their apical expression with their association to partner proteins. We will in particular focus on the situation of B<sup>0</sup>AT1 and B<sup>0</sup>AT3, that associate with members of the renin-angiotensin system (RAS), namely Tmem27 and angiotensin-converting enzyme 2 (ACE2), in a tissue specific manner.

The role of this association in relation to the formation of a functional unit related to Na<sup>+</sup> or amino acid transport will be assessed. We will conclude with some remarks concerning the relevance of this association to Hartnup disorder, where some mutations have been shown to differentially interact with the partner proteins.

**Keywords:** Amino acid transporters, B<sup>0</sup>AT1, Tmem27, ACE2, SLC6, Kidney, Small intestine, Renin Angiotensin System, Hartnup disorder

**Abbreviations:**

RAS : renin angiotensin system

SLC : solute carrier

HAT: heterodimeric amino acid transporters

SGLT1 : sodium dependant glucose transporter 1

MeAIB : methylaminoisobutyrate

Ang : Angiotensin

AT1R : Angiotensin II type 1 receptor

AT2R : Angiotensin II type 2 receptor

ACE : Angiotensin Converting Enzyme

MasR : G protein-coupled receptor encoded by the mas protooncogene

The description of amino acid transport has greatly evolved in the last 50 years, from the original transport systems description to the molecular identification of the transporters. Transport systems were classified according to the physico-chemical properties, stereospecificity, size and charge of the amino acids transported, as well as the ion or pH dependency and/or inhibition by (synthetic) amino acids.

Identification of the molecular correspondents of these systems in the early 90s was a great advance and allowed the development of the necessary tools to characterize the variability and the subcellular localization of these transporters.

A new development in the characterization of amino acid transporters has appeared through the increasing evidence of their association with partner proteins. It is reasonable to assume that the partner proteins are selected in an evolutionary coherent process in order to impact on transporter function and form a functional unit.

The following review will focus on SLC6 amino acid transporters, some of which have been shown to associate with partner proteins. The situation of these transporters is particularly interesting, as the partner proteins are tissue-specific and may thereby be linked to the etiology of Hartnup disorder. It is also interesting to note that these partner proteins were originally described because of their role in the renin angiotensin system (RAS) that is mainly known to regulate blood pressure and Na<sup>+</sup> reabsorption.

### **Amino Acid Transporters of the SLC6 Family**

The solute carrier family 6 (SLC6), is also called Sodium- and chloride-dependent neurotransmitter transporter family. It is formed by 21 members of about 600 amino acids and 12 transmembrane domains<sup>1</sup>, grouped by homology and divided in four branches (GABA, monoamine, amino acid I and amino acid II transporter branch) (Figure 1). The solutes transported by the members of this family range from neurotransmitters to osmolytes and amino acids. In contrast to channels, the transporters of the SLC6 family are never fully open, actively transport the substrates against a concentration gradient at a speed that is about 10'000 times slower than channels.

Most of the neurotransmitter transporters are expressed in the central and peripheral nervous system in the presynaptic membrane of neurons and can be the target of pharmacological manipulation. They are characterized by the rapid regulation of their insertion in the membrane and their high affinity transport (micromolar range). The osmolytes betaine and taurine are also transported by members of the Slc6 family and have important osmoregulation functions on kidneys and brain. We will focus our attention on the amino acid transporters of the SLC6 family. For the purpose of this paper, the amino acid transporters were grouped by the physico-chemical properties of their substrates in neutral, neutral and cationic and imino transporters. The amino acid

transporter members of the SLC6 family are mainly expressed in the central nervous system and epithelial cells. With the exception of B<sup>0</sup>AT1 (Slc6a19), they are high affinity transporters with sodium dependence (symporters) with the number of Na<sup>+</sup> varying from 1 to 3,<sup>2,3</sup> whereas chloride dependence is variable. The majority of the transporters localize to the plasma membrane with the exception of NTT4 (Slc6a17), which was characterized as a vesicular transporter (Table 1).

#### Neutral amino acids

##### B<sup>0</sup>AT1 (*Slc6a19*)

B<sup>0</sup>AT1 (*Slc6a19*) is the major neutral amino acid transporter in epithelial cells of kidney and small intestine. The sodium dependent transport of a broad range of neutral amino acids was functionally described as system B<sup>0</sup> much earlier than the identification of the transporter.<sup>4, 5</sup> The transporter was molecularly identified and characterized as a sodium and broad range neutral amino acids co-transporter with low affinity (millimolar range).<sup>6-8</sup> B<sup>0</sup>AT1 is only chloride sensitive but not dependent and does not transport biogenic amines or osmolytes.<sup>6, 7</sup> B<sup>0</sup>AT1 mRNA is highly expressed in kidney proximal tubule and small intestine where the protein localizes to the brush border membrane of small intestine enterocytes and the early portion of the proximal kidney tubules (S1).<sup>9, 10</sup> The transporter interaction with accessory proteins and its role in Hartnup disorder will be discussed in later sections.<sup>11-13</sup> A knock-out mouse model of B<sup>0</sup>AT1 has recently been described and these mice were shown to display decreased intestinal and renal neutral amino acid transport, the latter leading to neutral aminoaciduria similar to Hartnup subjects (discussed later on this review). Lower body weights and weight loss of mice under low (6%) and high (40%) protein diets and a blunted insulin response was observed.<sup>14</sup>

##### B<sup>0</sup>AT2/NTT7-3 (*Slc6a15*)

B<sup>0</sup>AT2 has 35-40% identity to the epithelial transporter B<sup>0</sup>AT1 (Slc6a19). Even though it was molecularly identified before B<sup>0</sup>AT1, its function remained unknown for many years. It has a particular substrate specificity for branched chain amino acids like L-isoleucine, L-valine, L-leucine, L-methionine, small amino acids like L-alanine and the imino acid L-proline, characteristics reminiscent of the IMINO system. The co-transport of amino acids with high affinity is sodium dependent and chloride sensitive. It was shown to be expressed in the brain (cortex, cerebellum and brain stem), kidneys, lungs, and testis.<sup>15, 16</sup> The function of this transporter is not completely understood. In the central nervous system, B<sup>0</sup>AT2 is expressed mainly in the neurons, whereas kidney localization of the transporter was not solved. The knock-out mice demonstrate lower L-leucine accumulation in synaptosomes with mild behavioral alterations and normal viability.<sup>17</sup>

### B<sup>0</sup>AT3 (*Slc6a18*)

B<sup>0</sup>AT3 shares 50% identity with B<sup>0</sup>AT1 in rodents and humans. The gene SLC6A18 is arranged in tandem with SLC6A19 (B<sup>0</sup>AT1) on chromosome 5p15 in human.<sup>10</sup> It is highly expressed in the apical membrane of the proximal tubules in the kidney and displays an axial arrangement complementary to B<sup>0</sup>AT1 along the proximal tubule, being higher in the more distal segments (S2, S3). Functionally, B<sup>0</sup>AT3 was shown to transport a broad range of neutral amino acids as for B<sup>0</sup>AT1, but with high affinity (micromolar range) in a sodium and chloride dependent manner. As for B<sup>0</sup>AT1, B<sup>0</sup>AT3 requires an accessory protein to be correctly expressed at the membrane.<sup>12, 18, 19</sup> B<sup>0</sup>AT3 appears to be required for the reabsorption of tubular amino acids leftover by B<sup>0</sup>AT1 in the early proximal kidney tubule segments or amino acids that have leaked back through the paracellular pathway.<sup>20</sup> In a knock-out mouse model of *slc6a18*, an abnormal excretion of several neutral amino acids was observed, especially glycine and L-glutamine. The mice otherwise develop normally and are viable but have been shown to develop high blood pressure under stress conditions.<sup>18, 21</sup> In humans, the connection between single nucleotide polymorphism, high blood pressure or myocardial infarction has been examined and remained inconclusive.<sup>22, 23</sup>

### Glyt1 (*Slc6a9*)

Glyt1 is expressed in brain, lung, kidney, small intestine, pancreas and liver.<sup>24, 25</sup> In brain, it is expressed in glial processes close to inhibitory neurons which use glycine as a neurotransmitter. When over-expressed in polarized epithelial cells, the isoforms Glyt1a and Glyt1b localize to the apical and basolateral membranes, respectively. It induces a high affinity (micromolar range) sodium and chloride dependent uptake of glycine.<sup>26</sup> Glyt1 knock-out mice suffer severe motosensory and respiratory deficits, and die shortly after birth.<sup>27</sup> Nothing is described about the role of Glyt1 deletion on epithelial cells of the kidney or small intestine.

### Glyt2 (*Slc6a5*)

Glyt2 is mainly expressed in brain, more precisely in axons and presynaptic terminals of inhibitory neurons that secrete glycine. When overexpressed in polarized epithelial cells it localizes to the apical membrane and like Glyt1, induces a high affinity (micromolar range) sodium and chloride dependent uptake of glycine.<sup>28</sup> The ablation of *slc6a5* gene in mice impairs high-affinity glycine uptake in brain areas with glycine-mediated transmission. The mice appeared to be normal at birth but die prematurely, displaying severe neuromotor alterations.<sup>29</sup>

### NTT4 (*Slc6a17*)

The so called neurotransmitter transporter-4 (NTT4), member 17 of the Slc6 family was also identified in the early 90's, but remained an orphan transporter for many years.<sup>30</sup> It has high identity (60%) with B<sup>0</sup>AT2 (Slc6a15), is sodium dependent but chloride independent as B<sup>0</sup>AT1 and 2 (Slc6a19 and 15, respectively). It transports L-proline, and neutral amino acids (L-alanine, glycine and L-leucine) like B<sup>0</sup>AT2 but with low affinity. It differs from the other members of the family by being a vesicular transporter rather than a plasma membrane transporter.<sup>31, 32</sup> It is expressed in brain, more specifically in glutamatergic and gabaergic neurons.<sup>30</sup> In the kidneys, it was shown to be highly expressed at the mRNA level in the medullary and cortical thick ascending limb and more distal nephron segments.<sup>33</sup> The function of this transporter is still not clear and a knock-out mouse model is not available yet.

#### Cationic and neutral amino acids

##### ATB<sup>0,+</sup> (*Slc6a14*)

ATB<sup>0,+</sup> is a sodium and chloride dependent high affinity transporter for almost all neutral and cationic amino acids. ATB<sup>0,+</sup>(*slc6a14*) shares moderate identity with B<sup>0</sup>AT1 (30% protein identity). The mRNA is highly expressed in brain, salivary and mammary glands, lungs and large intestine.<sup>34, 35</sup> A knock-out mouse model is not available yet.

#### Imino acids

##### SIT1 (*Slc6a20*)

SIT1 co-transporters with high affinity (micromolar range) L-proline, methylaminoisobutyrate (MeAIB), methyl-proline, and hydroxyproline with sodium and chloride and escapes  $\beta$ -alanine inhibition.

In mouse SIT1 is encoded by *Slc6a20a* (XT3s1) whereas the product of a second closely related gene *Slc6a20b* (XT3) does not lead to any uptake.<sup>36</sup> There is however only one gene in human. The mRNA is highly expressed in small intestine, kidney, lungs, spleen, thymus, testis, and brain. The protein localizes to the brush border membrane of the proximal tubules (S1=S2=S3) and enterocytes.<sup>10, 37</sup> SIT1, as B<sup>0</sup>AT1 and 3 (*Slc6a19* and *18*), may also functionally interact with accessory proteins. SIT1 transporter represents the molecular component of the previously described IMINO system. Other transporters, using proton as driving force (PAT1 and PAT2 or *Slc36a1* and *2*, respectively), were also described to transport imino acids and additionally glycine and  $\beta$ -alanine in intestine and kidney.<sup>36-40</sup> There is no knock-out mouse model for SIT1 (*slc6a20*) yet.

##### PROT (*Slc6a7*)

PROT is a specific L-proline transporter expressed in brain, more specifically in glutamatergic neurons. It is a high affinity sodium and chloride dependent transporter.<sup>41</sup>

## Partner Proteins

Some carriers were shown to interact with other proteins for appropriate localization/expression and/or function. The interaction guarantees the insertion of the transporter in the membrane and may modulate the activity or the supply of substrate. The amino acid transporters accessory partner proteins have been shown to be implicated in a variety of cellular or physiological functions such as cell surface antigens (CD98), endoplasmic reticulum proteins (GTRAP3-18 or 41), other amino acid transporters (TAT1-4F2hcLAT2 or ASCT2-4F2hc-LAT1) and the renin angiotensin system (RAS). The mechanisms of interaction are variable and will be discussed in the following section.

### Heavy chains and the heterodimeric amino acid transporters (slc3 family)

The interaction of amino acid transporters of the Slc7 family with type II glycoproteins of the Slc3 family is necessary for a correct targeting of the transporter-complex to the plasma membrane. The so called heterodimeric amino acid transporters (HAT) family is formed by an amino acid transporter or light chain (*Slc7* members) which interact with the surface antigen CD98/4F2hc or rBAT (related to  $b^{0,+}$  amino acid transporter). rBAT and 4F2hc are also called heavy chains, and are the only members of the solute carrier 3 family (*Slc3a1* and 2, respectively). To this day,  $b^{0,+}$ AT (*Slc7a9*) is the only member of the SLC7 family that was shown to interact with rBAT, while several transporters interact with 4F2hc (namely LAT1, LAT2,  $\gamma^+$ LAT1,  $\gamma^+$ LAT2, xCT, Asc-1 (*Slc7a5*, 8, 7, 6, 11 and 10, respectively)). rBAT and 4F2hc share 30% identity and target to different membrane compartments in polarized epithelial cells, rBAT being directed to the apical membrane and 4F2hc to the basolateral one.<sup>42</sup> The interaction of 4F2hc or rBAT with the HAT transporters was shown to be made via a disulfide bridge.<sup>43</sup>

When over-expressed together in cell line models, the heavy chain rBAT (*Slc3a1*) is mostly expressed as a complex with  $b^{0,+}$ AT (*Slc7a9*). When expressed alone, rBAT remains endoglycosidase H sensitive, is disposed by the ER-mannosidase-dependent ERAD pathway and degraded.<sup>44</sup> Recently, a trafficking signal on the transporter or light chain (*Slc7a9*) was suggested to define the fate of the complex. If the complex rBAT- $b^{0,+}$  is formed, rBAT will be able to leave the ER, go through full glycosylation and rBAT- $b^{0,+}$  will reach the membrane as an active complex. The deletion or mutation of the C-terminal signal of  $b^{0,+}$ AT (*Slc7a9*) does not prevent complex formation, but leads to the formation of an inactive complex.<sup>45</sup> Interestingly, mutations in the gene encoding the heavy chain or accessory protein rBAT (*Slc3a1*) as well as in its interacting light chain  $b^{0,+}$ AT (*Slc7a9*) are associated with the autosomal disease cystinuria. Mutations on the gene encoding 4F2hc (*Slc3a2*), which associates with several members of the Slc7 family, are not connected to any physiological anomaly.<sup>46</sup>



#### Modulating interacting proteins (GTRAP3-18 or 41, negative modulators of Slc1 family members)

Members of the glutamate and aspartate transporters from the solute carrier family 1 (*Slc1*) were shown to be modulated by the Glutamate Transport Associated Protein for EAAT3 (*Slc1a1*) and EAAT4 (*Slc1a6*) (GTRAP3–18 or 41). GTRAPs are structurally homologous to the Ras superfamily in their secondary structure and molecular weight, but lack a GTP-binding consensus motif. GTRAP3-18 is an integral endoplasmic reticulum (ER) membrane protein with four transmembrane domains and cytosolic N and C termini.<sup>47</sup> GTRAP3–18 interacts with the carboxy-terminal end of the transporter and regulates the function of EAAT3/EAAC1 (*Slc1a1*) by delaying its exit from the ER.<sup>47</sup> GTRAP3-18 is expressed in the same tissues EAAT3 is present in,<sup>48</sup> and the interaction of EAAT3 in the ER with GTRAP3-18 oligomer protein may prevent the complex from exiting as cargo proteins to the Golgi.

#### Functional interaction: TAT1 / LAT2-4F2 and ASCT2 / LAT1-4F2

The functional interaction of antiporter transporters with other carriers has been shown to provide substrates to one of the carriers and to control cell volume and apoptosis. The interaction is only functional, no physical interaction was observed between the transporters involved. The first described interaction of this kind was among transporters expressed on the basolateral membrane of epithelial cells of the kidney proximal tubules involving an amino acid uniporter and an amino acid antiporter. The substrates transported from the cytoplasm to the extracellular compartment of the cells by the uniporter TAT1 (*Slc16a10*) can be used as substrate of the antiporter or obligatory exchanger LAT2-4F2hc (*Slc7a8-Slc3a2*) and possibly by  $\gamma$ -LAT1-4F2hc (*Slc7a7-Slc3a2*).<sup>49</sup> The uniporter has a very narrow specificity and low affinity, transporting only aromatic amino acids. The exchanger LAT2-4F2hc can import the effluxed aromatic amino acids outside of the cells in exchange for cytoplasmic neutral amino acids. The aromatic amino acid can again be recycled by the uniporter, triggering the efflux of neutral amino acids by the exchangers, favoring re-absorption. Whether this interaction triggers a cascade of events in the epithelial cells, which might control the transport by changing the expression of the transporter or its presence at the membrane has not been elucidated yet.

The interaction between the symporter ASCT2 (*Slc1a5*) and the antiporters of the HAT family LAT1-4F2hc (*Slc7a5-Slc3a2*), was shown to trigger an activation cascade upstream of the mTOR pathway that was independent of insulin.<sup>50</sup> L-glutamine transported into cells by ASCT2 triggers the transport of essential amino acids by LAT1-4F2hc. The high intracellular concentration of L-glutamine and the later accumulation of essential amino acids into the cytoplasm activate rapamycin-sensitive mTOR complex 1 (mTORC1). This two steps amino acid exchange is necessary to activate the downstream cascade with phosphorylation and activation of the 70 kDa ribosomal protein S6 kinase (S6K), induce cell growth and inhibit autophagy. This work proposes a functional interaction between amino acid

transporters upstream of mTOR that is necessary to activate the pathway. In addition, these results suggest that the expression levels of the transporters may also be related to cell division, survival and recovering of the tissue when exposed to different diets or after injury.

#### Solute transport and the renin angiotensin system

Neutral amino acid transporters of the SLC6 family have recently been shown to rely on the expression of Tmem27 and the angiotensin-converting enzyme 2 (ACE2), two members of the RAS.

The RAS is critical in the regulation of blood pressure homeostasis, electrolyte balance and extracellular volume. The main effector of the RAS is the key component angiotensin (Ang) II that mediates the majority of its cardiac and renal actions by binding Ang II type 1 receptor (AT1R).<sup>51, 52</sup>

Responses include vascular smooth muscle contraction, enhanced myocardial contractility, aldosterone secretion, catecholamine release from medulla and sympathetic nerve endings, adrenergic stimulation, increased sympathetic nervous system activity, renal sodium reabsorption and thirst and salt appetite stimulation.<sup>53, 54</sup> Ang II also binds to Ang II type 2 receptor (AT2R), inducing a counter regulatory vasodilation through the stimulation of (NO)/cGMP, mediated by bradykinin release (aorta, heart, kidney), but also growth inhibition and apoptosis promotion.<sup>55</sup>

In recent years, it has become clear that the RAS functions at two levels, the classical systemic RAS, as well as the local, tissue or intrinsic RAS.

Systemic RAS leads to the generation of circulating Ang II through a series of cleaving events. The decapeptide precursor angiotensinogen (AGT) produced by the liver is first cleaved by the protease renin secreted by kidney juxtaglomerular cells in response to low effective circulating volume to the octapeptide Ang I. Ang I is then itself cleaved to Ang II by the dipeptidyl carboxypeptidase angiotensin converting enzyme (ACE) that is highly expressed on the surface of endothelial cells of the pulmonary circulation (Figure 2a).

#### ACE2 (ACEH)

The classical RAS view was complemented by the discovery of ACE2, an ACE homologue sharing 41.8% sequence identity with its extracellular domain that was originally identified in a screen for metalloproteases as well as a human heart failure ventricle library (Figure 2b).<sup>56, 57</sup> ACE2 was also found to play a role in the regulation of Ang II levels, although in contrast to ACE, the catalytic site of ACE2 only displays a single HEMGH zinc-binding catalytic domain. Another difference relies in the fact that ACE2 functions as a carboxypeptidase, which cleaves the terminal hydrophobic/basic amino acid following a L-proline ( $\pm$  1-3 residues)<sup>58</sup>.

ACE2 expression was measured in kidney, heart, testis, vascular smooth muscle cells, lung, liver, small intestine, placenta and brain, and localizes to the apical membrane in polarized cells.<sup>59</sup>

Expression at the membrane is down regulated after extracellular shedding by the ADAMs family of zinc metalloproteinases,<sup>60</sup> and by binding of the SARS coronavirus.<sup>61, 62</sup>

The crucial role of ACE2 in the RAS relies on its ability to cleave Ang I and Ang II to Ang (1-9) and Ang (1-7), respectively (Figure 2a).<sup>56, 57</sup> The difference in protease target recognition is essential for the opposing functions of ACE and ACE2. Indeed, cleavage of Ang II by ACE2 not only decreases its bioavailability but also leads to the formation of Ang (1-7), that can bind to the G protein-coupled receptor encoded by the mas proto-oncogene (MasR), opposing AT1R-mediated vasoconstriction and cellular proliferation.<sup>63, 64</sup>

Furthermore, ACE2 is involved in cardiovascular and renal (patho)physiology, as well as in diabetes, acute lung disease and pregnancy.<sup>59</sup>

#### Tmem27 (Collectrin)

Tmem27 or Collectrin is a homologue of ACE2 that shares 47.8% identity with its non-catalytic extracellular, transmembrane and cytosolic domains.<sup>65</sup> Tmem27 was originally described as dramatically up regulated in 5/6 nephrectomized mouse kidney (NX-17) in a screen to identify genes involved in glomerular hyperfiltration and hypertrophy.<sup>66</sup> The *tmem27* gene is located next to *ace2* on chromosome Xp22 and encodes a 222 amino acid protein with a signal peptide that represents a 32 kDa membrane glycoprotein with a single transmembrane segment (Figure 2b).<sup>65</sup> Tmem27 has been shown to be expressed in liver, lung, endocrine pancreas and kidney proximal tubule brush border membrane and collecting duct, hence the misleading name Collectrin.<sup>12, 13, 67, 68</sup>

Given the lack of Tmem27 catalytic activity, it does not seem to play a role in the classical RAS although an implication in the development of salt sensitive hypertension has been suggested.<sup>69</sup>

Tmem27 has been shown to bind proteins involved in intracellular and membrane protein trafficking and ciliary movement such as  $\gamma$ -actin-myosin II-A, snapin, SNAP-25, and polycystin-2-polaris complexes. This may constitute the link between Tmem27 and its implication in glucose-stimulated insulin exocytosis and and/or  $\beta$ -cell mass in pancreas,<sup>68, 67</sup> as well as renal collecting duct primary cilium formation and polycystic kidney disease.<sup>70</sup>

As described more in detail in later paragraphs, Tmem27 has also been shown to be critical for the correct expression of amino acid transporters in kidney.<sup>12, 13</sup>

#### Local RAS

The notion of local, intrinsic or tissue RAS has emerged in recent years in complement to the general circulating RAS. Tissues exhibiting expression of some RAS components include the lung, pancreas, liver, intestine, heart, kidney, vasculature, carotid body and adipose tissue, as well as the nervous, reproductive and digestive systems.<sup>71, 72</sup> Local RAS action has been shown to be implicated in a wide

variety of functions distinct from the circulating RAS such as cell growth, anti-proliferation and apoptosis, reactive oxygen species (ROS) generation, pro-inflammatory and pro-fibrogenic actions, hormonal secretion, and also nutrient transport.<sup>53, 73, 74</sup>

#### Role of local ACE2

The kidney is particular in the regulation of its local RAS, as it expresses all the RAS components from renin to the AT receptors. The regulation of local Ang II levels was not always clear as it is difficult to discriminate locally synthesized components from those provided by the circulation and because renal RAS is compartmentalized between tubular, interstitial and intracellular systems.

Cleavage of Ang I to Ang II is mainly performed by ACE that, in kidney, mostly localizes to the brush border membrane of the proximal tubule in human, in contrast to rats that also display localization on vascular endothelial cells and the basolateral side.<sup>75</sup> The resulting levels of Ang II are up to a 100 times higher than in plasma, which argues in favor of a local RAS as these levels cannot be achieved by the systemic supply alone.<sup>76</sup>

Physiological actions of Ang II occur in the proximal tubule and further along the nephron in the distal segments and the collecting duct.<sup>77</sup> The Ang II locally produced favors Na<sup>+</sup> retention through the modulation of renal hemodynamics (decreased renal blood flow and glomerular filtration rate, afferent and efferent arteriole constriction and enhanced tubuloglomerular feedback) and increased Na<sup>+</sup> transport.

The increase of Na<sup>+</sup> re-absorption induced by Ang II in proximal and distal tubules is mediated by activation of AT1R.<sup>78</sup> In the proximal tubules, Ang II favors the expression of the H<sup>+</sup>-ATPase expression, thereby increasing transcellular sodium reabsorption through the Na<sup>+</sup>/H<sup>+</sup> exchanger.<sup>79-83</sup>

As described previously, renal SLC6 members co-transport Na<sup>+</sup> and thereby participate in Na<sup>+</sup> re-absorption. A possible hypothesis is that the interaction of these Na<sup>+</sup> co-transporters with ACE2, the enzyme counter regulating Ang II amounts and effects, could be a fine tuning mechanism to control Na<sup>+</sup> reabsorption. The functional link would explain the dependence of these amino acid transporters on elements of the RAS. Such a hypothesis has however not been tested until now, and this mechanistic link might have been lost through evolution by the shift of the partner protein from ACE2 to Tmem27 in the kidney.

The role of local RAS in small intestine and the expression of RAS components in enterocytes have led to the investigation of its impact on nutrient absorption.<sup>84</sup> Local intestinal RAS system has also been shown to regulate sodium dependent glucose transporter 1 (SGLT1, *Slc5a1*) expression in rat small intestine. There, SGLT1-mediated glucose transport in intestinal ring segments was decreased by exposure to Ang II through transporter down regulation, which was reversible by AT1R antagonist

treatment.<sup>84</sup> Additionally, ACE2 gene expression in Caco2 cells was induced by high glucose treatment.<sup>85</sup>

We could however demonstrate that the expression of ACE2 on the apical membrane of enterocytes as well as Tmem27 in kidney is necessary for the expression of amino acid transporters. Members of the Slc6 family transporters, more specifically the neutral amino acid transporters B<sup>0</sup>AT1 and 3 (Slc6a19 and 18) and imino transporter SIT1 (Slc6a20) interact with Tmem27 and ACE2, but not with ACE. The interaction mechanism of the transporters with the accessory proteins is not completely elucidated, but has been speculated by several groups.

### **Neutral amino acid transporters associate with Tmem27 and ACE2**

#### Tmem27 and ACE2 partner proteins

B<sup>0</sup>AT1 and B<sup>0</sup>AT3 were first shown to rely on Tmem27 expression to be correctly expressed at the brush border membrane of renal proximal tubule cells. Indeed B<sup>0</sup>AT1 and B<sup>0</sup>AT3 were strongly down regulated in the kidneys of *tmem27* knock-out mice which led to massive amounts of amino acids lost in the urine.<sup>12, 13</sup>

Discovery of ACE2 as a second partner protein for B<sup>0</sup>AT1 arose when the transporter was observed to be normally localized at the membrane of small intestine enterocytes of *tmem27* knockout mice, but absent from knock-out mice for its homologue ACE2.<sup>9, 11</sup>

#### Partner in kidney

Tmem27's role as a partner protein of amino acid transporters was revealed by gene ablation. Two independent groups generated *tmem27* (*coll*) knock-out mice and observed a strong generalized neutral aminoaciduria, but no glucosuria or phosphaturia. Kidney morphology of *tmem27* knock-out mice was normal and the mice were fertile and viable.<sup>12, 13</sup> Analysis of amino acid transporters expression in kidney showed normal RNA but abnormal protein expression in proximal tubules with reduced transport function. The membrane expression of the neutral amino acid transporters of the Slc6 family, B<sup>0</sup>AT1, B<sup>0</sup>AT3 (*Slc6a19* and *18*), the imino transporter SIT1 (*Slc6a20*) but also Slc1 L-glutamate and L-aspartate transporter EAAT3 (*Slc1a1*) were abolished or strongly reduced.<sup>12, 13</sup> The accumulation of neutral amino acids was shown to be decreased in renal brush border membrane vesicles of *tmem27* knock-out mice. To confirm the interaction, the function of the transporters was assayed in the *Xenopus laevis* heterologous expression system. The presence of Tmem27 increased transporter function and accelerated expression kinetics. Interestingly, the expression of human B<sup>0</sup>AT1 with human Tmem27 induced a stronger effect on B<sup>0</sup>AT1 function than the mouse Tmem27 ortholog. Tmem27 orthologs share 84% identity and the dissimilar residues are distributed in all segments of the protein. The C-terminal domain of Tmem27 appear to be involved in the interaction

with B<sup>0</sup>AT1 according to our split ubiquitin experiments (unpublished data), but the role of this domain or of the specific residues were not studied yet.

The association between Tmem27 and B<sup>0</sup>AT1 may occur through non-covalent interactions in contrast to Slc3 (4F2hc or rBAT) and Slc7 members. The proteins co-localize at the apical membrane and the surface expression of B<sup>0</sup>AT1 is increased by Tmem27 presence *in vitro* and *in vivo*. *In vitro*, the surface expression increase suggests a chaperone function, but the impact on the function was much sturdier than the effect seen on the surface expression of the protein. This would suggest that the association may also impact transporter function, possibly by increasing the cycling rate.

#### Other members of the SLC6 family interacting with Tmem27 in kidney

The function of other members of the Slc6 family was also modulated by Tmem27. B<sup>0</sup>AT3 (*Slc6a18*) and SIT1 (*Slc6a20*) membrane expression were decreased in the brush border of *tmem27* knock-out mice. Accordingly, the neutral aminoaciduria observed in these mice is broader than that typically observed in humans with Hartnup disorder that display mutations in B<sup>0</sup>AT1, and also extends to L-proline suggesting the additional implication of the IMINO system. The loss of neutral amino acids in the urine, is probably caused by the decrease of both B<sup>0</sup>AT3 and B<sup>0</sup>AT1 (*Slc6a18 and 19*), however L-proline is poorly transported by B<sup>0</sup>AT1 and 3.<sup>7, 12, 18, 86</sup> This suggests that L-proline loss in urine might be due to the decreased expression of SIT1 (*Slc6a20*). Exogenous expression of B<sup>0</sup>AT3 with Tmem27 in *Xenopus laevis* oocytes generates divergent results. Our own experiments showed that the expression of B<sup>0</sup>AT3 with Tmem27 (mouse or human orthologs) did not result in a substantial increase of transport function or membrane expression.<sup>18</sup> However, Vanslambrouck and colleagues showed a more than 10 fold increase in function with the co-expression of mouse B<sup>0</sup>AT3 and Tmem27.<sup>19</sup> To characterize the transport in heterologous expression system we overexpressed the transporter with the other member of the RAS system, ACE2 (Figure 3). With this approach, we could characterize the function of mouse B<sup>0</sup>AT3 that had remained an orphan transporter until then. The protein interaction mechanism is unknown. It is interesting that B<sup>0</sup>AT3 readily reaches the membrane when overexpressed alone. In the presence of ACE2, we observed a slight increase of protein expression at the membrane, but most importantly, the function was revealed. The protein migrates equally when expressed in *Xenopus laevis* alone or in the presence of ACE2 or Tmem27. In the native tissue, the transporter most likely interacts with Tmem27.<sup>10</sup>

The axial gradient of B<sup>0</sup>AT3 along the proximal tubule corresponds to that of ACE2, *i.e.* higher expression in the later segments (S3), while Tmem27 expression follows the opposite trend. Interestingly, the ablation of *ace2* neither interferes with B<sup>0</sup>AT3 expression on the apical membrane of the proximal tubules nor with the function of the transporter.

B<sup>0</sup>AT3 knock-out mice present mild aminoaciduria characterized by high excretion of glycine, L-glutamine, L-methionine, L-alanine, L-threonine, L-serine, branched chain and aromatic amino acid. The urinary analysis of the *ace2* knock-out mice showed no aminoaciduria. Knowing that the ablation of *tmem27* induces a massive neutral aminoaciduria and decreases B<sup>0</sup>AT3 expression, it seems that Tmem27 is the partner of B<sup>0</sup>AT3 in the kidney.

#### Partner in small intestine

The transporter B<sup>0</sup>AT1 (*Slc6a19*) is highly expressed not only in kidney, where it interacts with Tmem27, but also in small intestine where Tmem27 is absent. We hypothesized that the transporter interacts with another RAS member expressed in the small intestine, namely ACE2. Using the knock-out mouse model and an overexpression system, we could demonstrate that ACE2 is indeed the partner of B<sup>0</sup>AT1 in small intestine. The two proteins co-localize at the apical membrane of enterocytes of wild type mice and humans, and ACE2 could be co-immunoprecipitated with B<sup>0</sup>AT1 antibody from brush border membrane preparations. In *ace2* knock-out mice, expression of B<sup>0</sup>AT1 is completely abolished in the brush border of small intestine, but normal in kidney. Additionally, Na<sup>+</sup> dependent transport of neutral amino acids was decreased in *ace2* knock-out mice small intestine. The interaction was confirmed by the overexpression model, where co-expression with ACE2 increased transporter membrane expression and function. Mouse and human ACE2 orthologs induced similar increases of transporter function unlike Tmem27, where only the human ortholog induced an effect on human B<sup>0</sup>AT1.<sup>9, 11</sup>

Another transporter apparently having two tissue specific accessory proteins is SIT1 (*Slc6a20*). In the *ace2* knock-out mice we observed a decrease in sodium dependent, pH independent L-proline transport (unpublished results).

The interaction mechanism of B<sup>0</sup>AT1 (*Slc6a19*) with ACE2 is also not known. Based on the analysis of several B<sup>0</sup>AT1 mutants, we postulate that the absence of ACE2 may cause a decrease of transporter insertion or stability at the membrane.

The physiological importance of this interaction could be explained from the transporter point of view. The ACE2 carboxypeptidase activity would provide substrate for the transporter with which it is interacting.<sup>9</sup> This theory would explain the symbiosis between B<sup>0</sup>AT1 and ACE2 in the small intestine, since the carboxy amino acids released by ACE2 cleavage are mostly neutral amino acids, but also cationic.<sup>87</sup> A catalytic dead mutant of ACE2 however still had the same effect on B<sup>0</sup>AT1 function. The *slc6a19* knock-out model (B<sup>0</sup>AT1) has normal expression of ACE2 and Tmem27 suggesting that transporter ablation has no impact on RAS system members.<sup>88</sup> We can therefore not conclude if the interaction also has a positive effect for the enzyme. Evidence however suggests that this is not the case, as the enzyme reaches the membrane in organs where the amino acid transporter is not

expressed, suggesting the interaction is important for the transporter but not crucial for RAS members.

#### B<sup>0</sup>AT1, Tmem27, ACE2 and Hartnup disorder

The aminoaciduria observed in *tmem27* knock-out mice was shown to be caused by the reduction of amino acid reabsorption, and resembles the one observed in Hartnup disorder. Namely, elevated urinary excretion of neutral amino acids, absence of glucosuria or phosphaturia, and normal plasma amino acids levels. Neutral aminoaciduria is a hallmark of Hartnup disorder, as it is observed in all patients and is the first diagnostic of the disorder. Under certain conditions, patients may also additionally develop symptoms resembling pellagra (including light-sensitive dermatitis), intermittent cerebellar ataxia and psychosis-like symptoms.<sup>89</sup>

Hartnup disorder is caused by mutations in gene *SLC6A19* (chromosomal locus 5p15.33) encoding the B<sup>0</sup>AT1 transporter. Even though B<sup>0</sup>AT1 is the major neutral amino acid transporter in kidney and small intestine, patients develop normally and may present symptoms, other than the aminoaciduria, only under stress conditions (infections, malnutrition, etc.). The aminoaciduria is attributed to the defective transporter in kidney, but the impact of the transport defect on intestinal absorption and its connection to pellagra or neurological manifestations is still unclear.

The first mutation of the *SLC6A19* gene (B<sup>0</sup>AT1) was identified in members of the British Hartnup family, in which the disorder was originally described.<sup>90</sup> Twenty-one additional mutations were further described to cause the disease, including deletions, missense, nonsense and splice site mutations on the gene *SLC6A19*, as depicted in Figure 4.<sup>90-93</sup> Most of the patients are compound heterozygous which could partially contribute to the variable symptoms observed in patients (Table 2).

Knowing that B<sup>0</sup>AT1 interacts with Tmem27 in kidney and ACE2 in small intestine, a natural question is the role of these accessory proteins on the phenotype of the disorder. Both the *ace2* and the *tmem27* genes are located on the X chromosome (Xp22). So far, no X-linked recessive Hartnup or other neutral aminoaciduria disorders have been described. This suggests that mutations on the accessory protein are not the cause of the disorder.

On the other hand, the mutated transporter may interact differently with the accessory proteins, and show a tissue dependent role. The mutations causing Hartnup disorder are distributed all over the protein. Using the crystallized structure of the Slc6 bacterial homologue LeuT<sub>aq</sub>,<sup>94</sup> we modeled B<sup>0</sup>AT1 and could identify the sites where the mutations are possibly localized (Figure 4). The domain responsible for the interaction with the accessory proteins is not yet known, but some important regions and residues, like sodium and substrate binding domain or domains which undergo



conformational changes during the transport cycle, were identified based on LeuT<sub>aq</sub> and previous cysteine scan studies.

The expression of B<sup>0</sup>AT1 containing Hartnup causing mutations with Tmem27 or ACE2 showed different effects on function and membrane expression of the transporter (Table 3). From the mutated transporters analyzed, we could identify an intriguing class of mutants (D173N and P265L) which differentially interacted with the two tissue-specific accessory proteins such that their function is stimulated only by the intestine-specific associated protein ACE2. The D173N allele was identified in different unrelated Hartnup pedigrees and displays a high heterozygote frequency in healthy Caucasians (1:122 healthy individuals).<sup>95</sup> The interaction with ACE2 might allow the “survival” of this frequent allele by increasing the amino acid absorption in the individuals bearing the mutation. Unfortunately, no data on the intestinal absorption of patient bearing the D173N mutation is available. Another complicating factor would be the heterozygosis of the patients. Until now, only 3 D173N homozygote mutant individuals have been identified in American and Australian pedigrees.

A second class of mutants corresponds to those which are not stimulated by either associated protein (R240Q and A69T). R240Q interacts with the accessory protein, but there is no increase of surface appearance upon co-expression with ACE2 or Tmem27 when compared to the transporter expressed alone. In the case of A69T, the surface expression is increased. The results suggest a different fate for these mutations. R240Q may impact on the interaction with accessory proteins, whereas A69T, which is localized in a domain that undergoes conformational changes during the transport, reaches the membrane as a complex but cannot work properly.<sup>9, 11, 96</sup>

The majority of the mutations tested (R57C, L242P, E501K, G93R and P579L) are dead mutants. They do not reach the membrane or display any measurable function. Finally, some mutations have not been assayed for the interaction with the tissue accessory proteins yet.

The phenotype of Hartnup patients has been shown to be very variable. One of the reasons for the variation observed can be the nutritional status and diet of the subjects and their environment. Another cause of variation may be the high frequency of compound heterozygous. Additionally, B<sup>0</sup>AT1 mutants can interact differentially with tissue specific accessory proteins, favoring the absorption of amino acids in the intestine and might also contribute significantly to the phenotypic heterogeneity among individuals with Hartnup disorder.

## **Conclusion and Outlook**

The work on amino acid transporters of the SLC6 family and their tissue-specific partner proteins has provided critical information concerning the modulation of amino acid transporter function, and the possible physiological relevance of such an association in the context of Ang II sensitivity. With this

information, new questions waiting to be solved have emerged concerning the exact beginning and extent of this association, as well as the mechanism guiding the selection of the Tmem27 partner protein in kidney, rather than ACE2 that is also expressed in that organ. Additionally, questions related to the regulation of the expression and function of the transporters and their accessory proteins by diet, gender, hormones, age and diseases are crucial and are just starting to be investigated. These questions will allow us to understand the complex mechanism of amino acids homeostasis maintenance and its relevance for several processes in the body distinct from the basic energy supply and protein synthesis. After the transporter cloning era, we predict an exciting translational era to answer old physiological questions related to amino acids transport.

Legends:

**Figure 1: Phylogenetic tree of human and mouse Slc6 family.** Protein sequences of the human and mouse Slc6 family members were aligned and a phylogenetic tree was constructed. The *Phylogeny.fr* platform was used to construct the tree.<sup>97, 98</sup> The members are divided in **GABA** (white, continuous line): hGAT1/*SLC6A1*, P30531; hGAT2/*SLC6A13*, Q9NSD5; hGAT3/*SLC6A11*, P48066; hTAUT/*SLC6A6*, P31641; hCT1/*SLC6A8*, P48029; hBGT1/*SLC6A12*, P48065; *SLC6A10* hypothetical protein, Q6ZUB2; mGAT1/*slc1a1*, P31648; mGAT2/*slc6a13*, P31649; mGAT3/*slc6a11*, P31650; mbetaine, GABA/*slc6a12*, Q8VCS9; mCT1/*slc6a8*, Q8VBW1; mTAUT/*slc6a6*, O35316. **Monoamines** (white, dashed line): hNET/*SLC6A2*, P23975; hDAT/*SLC6A3*, Q01959; hSERT/*SLC6A4*, P31645, mNET/*slc6a2*, O55192; mDAT/*slc6a3*, Q61327; m5HTT/*slc6a4*, Q60857. **Amino acids I** (gray, dashed line): hGlyt2/*SLC6A5*, Q9Y345; hGyt1/*SLC6A9*, P48067; hPROT/*SLC6A7*, Q99884; hB0+/*SLC6A14*, Q9UN76; mGlyt2/*slc6a5*, B2RQX9; mGlyt1/*slc6a9*, P28571; mPROT/*slc6a7*, Q6PGE7; mB<sup>0+</sup>/*slc6a14*, Q9JMA9. **Amino acids II** (orphans, gray, continuous line): hB<sup>0</sup>AT2/*SLC6A15*, Q9H2J7; hNTT5/*SLC6A16*, Q9GZN6; hNTT4/*SLC6A17*, Q9H1V8; hB<sup>0</sup>AT3/*SLC6A18*, Q96N87; hB<sup>0</sup>AT1/*SLC6A19*, NP\_001003841.1; hSIT1/*SLC6A20a*, Q9NP91; mB0AT2/*slc6a15*, Q8BG16; mslc6a16, XM\_355900.5; mNTT4/*slc6a17*, Q8BJI1; mB<sup>0</sup>AT1/*slc6a18*, O88576; mB<sup>0</sup>AT1/*slc6a19*, Q9D687; mSIT1/*slc6a20a*, NP\_035861.2; mXT3/*slc6a20b*, O88575.

**Figure 2: Renin angiotensin system and its members.** a) Schematic representation of the renin-angiotensin (RAS) system. The main role of the system involves ACE and the production of the octapeptide Angiotensin II. ACE2 role was suggested to be involved in the counterbalance effect of Angiotensin II and ACE activity. b) ACE2 and Tmem27 share high identity in the C-terminal domain. ACE2 and Tmem27 share identity in the C-terminus (white, shaded), whereas ACE and ACE2 in the N-terminal extracellular domain (grey, vertical lines). Somatic ACE (sACE), ACE2 and Tmem27/Collectrin are type I integral proteins with a signal peptide in the N-terminal, transmembrane, and short C-terminal domains. ACE2 and ACE have 1 or 2 zinc-binding motif (HEMGGH) (bm) in the extracellular domain. sACE is encoded by a gene located on human chromosome 17, while ACE2 and Tmem27 on the X chromosome. Testis ACE was omitted from the schematic representation. AT1R, Angiotensin type 1 receptor; AT2R, Angiotensin type 2 receptor; MasR, G protein coupled Mas proto-oncogene receptor.

**Figure 3: ACE2 and TMEM27, but not ACE, functionally interact with B<sup>0</sup>AT3 and B<sup>0</sup>AT1.** Mouse B<sup>0</sup>AT3 and 1 (*Slc6a18* and *19*, respectively) were expressed in *Xenopus laevis* oocytes alone or in combination with mouse Tmem27, ACE2 or ACE. Transporter alone (empty bars), co-expression with: Tmem27 (gray bars), ACE2 (checkered bars), ACE (solid bars). 10 ng of each cRNA was injected, and the transport of L-isoleucine (100  $\mu$ M for B<sup>0</sup>AT3 and 1 mM B<sup>0</sup>AT1 for 30 minutes) measured after 6 days expression. The low effect of Tmem27 in mouse B<sup>0</sup>AT1 after long expression was previously observed.<sup>11</sup> ACE expression in *tmem27* and *ace2* knockout is normal, supporting the hypothesis that ACE is not an accessory protein even though in oocytes injected with B<sup>0</sup>AT1 the transport was increased.

**Figure 4: Mutations in the SLC6A19 gene causing Hartnup disorder.** The homology model of human B<sup>0</sup>AT1 transport was based on the crystal structure of LeuT<sub>Ad</sub> (NP\_214423) as a template using the I-TASSER server.<sup>94, 99</sup> The pdb file was visualized using Pymol (DeLano Scientific LLC, Palo Alto, CA). The transporter is oriented with the top facing the lumen of the renal proximal tubule or small intestine and the bottom facing the intracellular compartment. The mutations are from refs.<sup>11, 90-93, 100, 101</sup>

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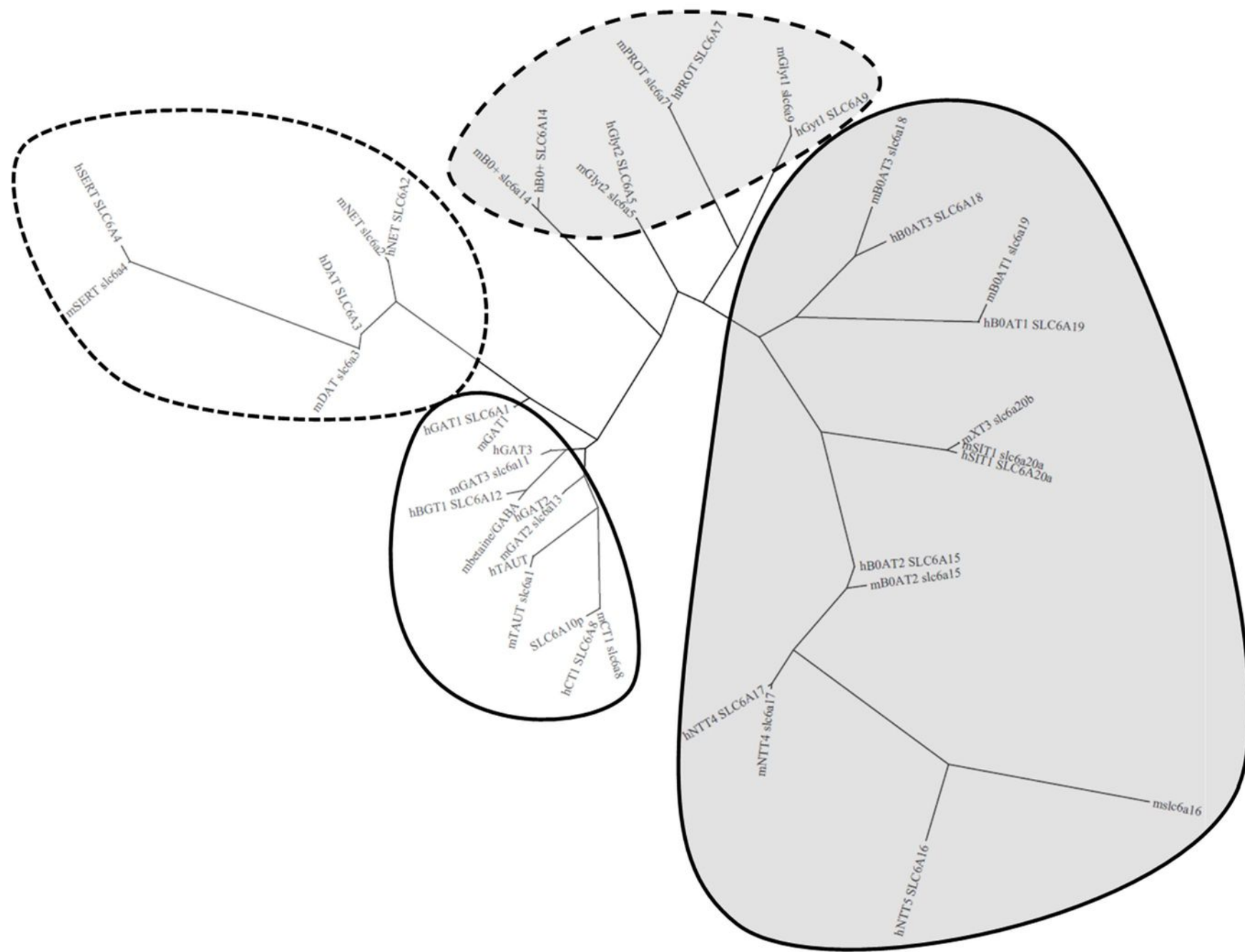
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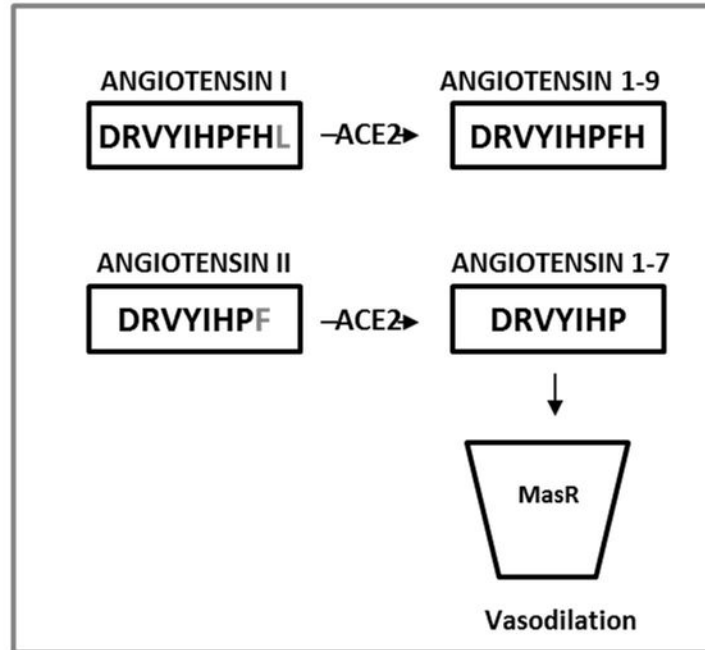
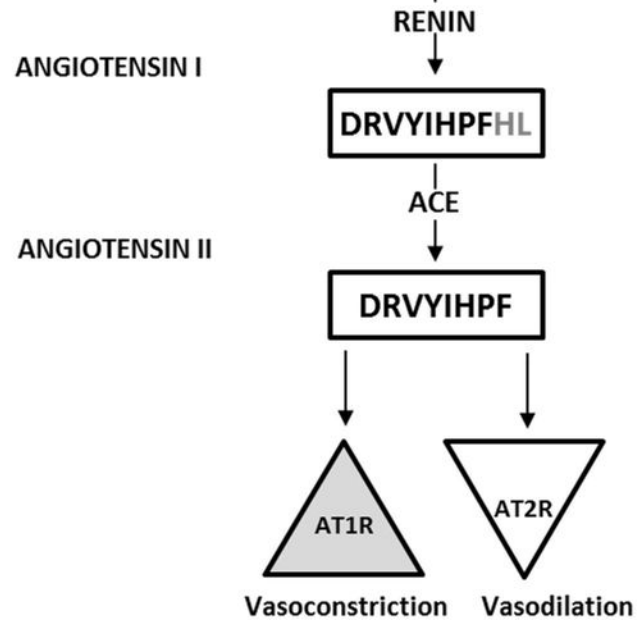
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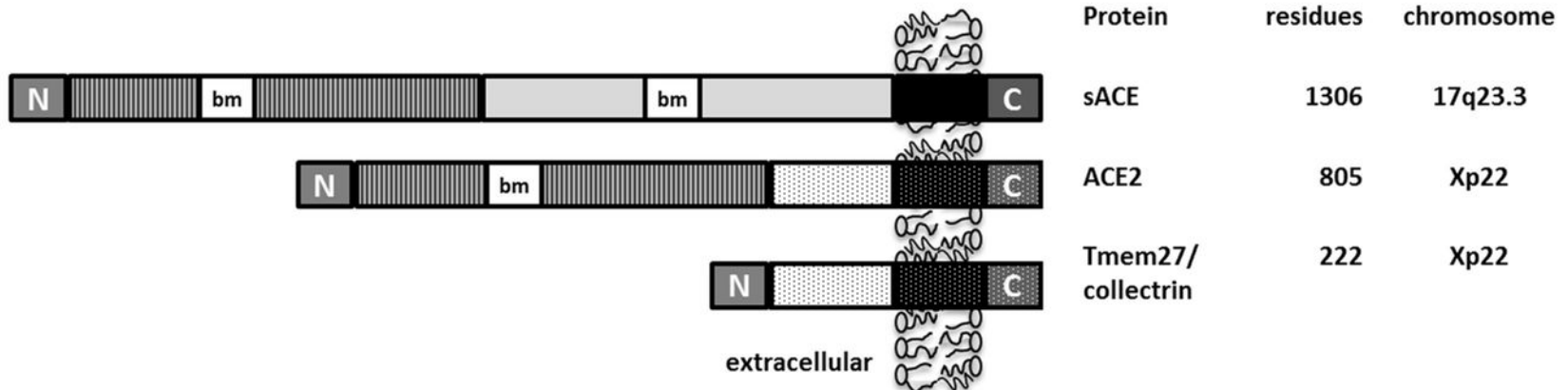
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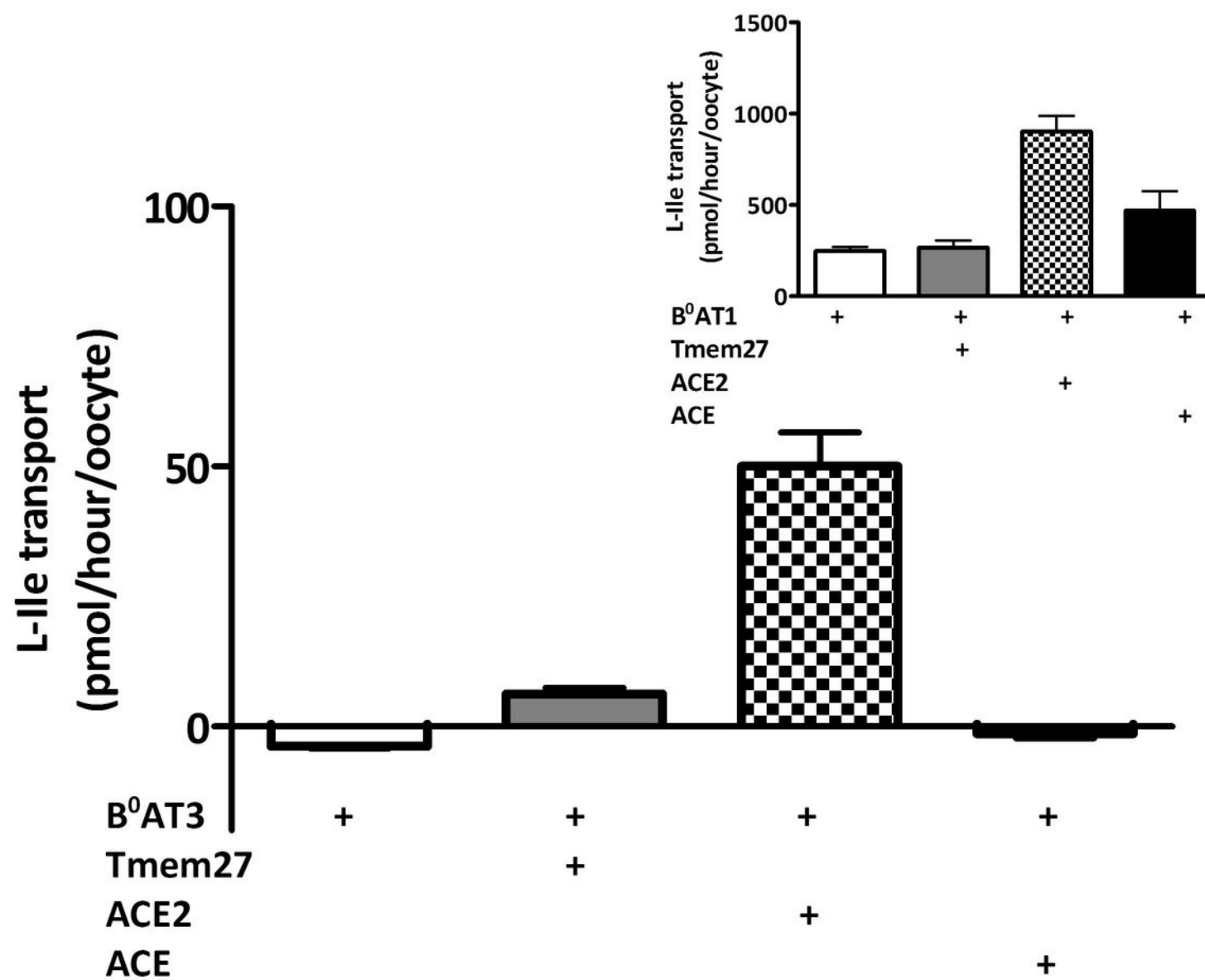
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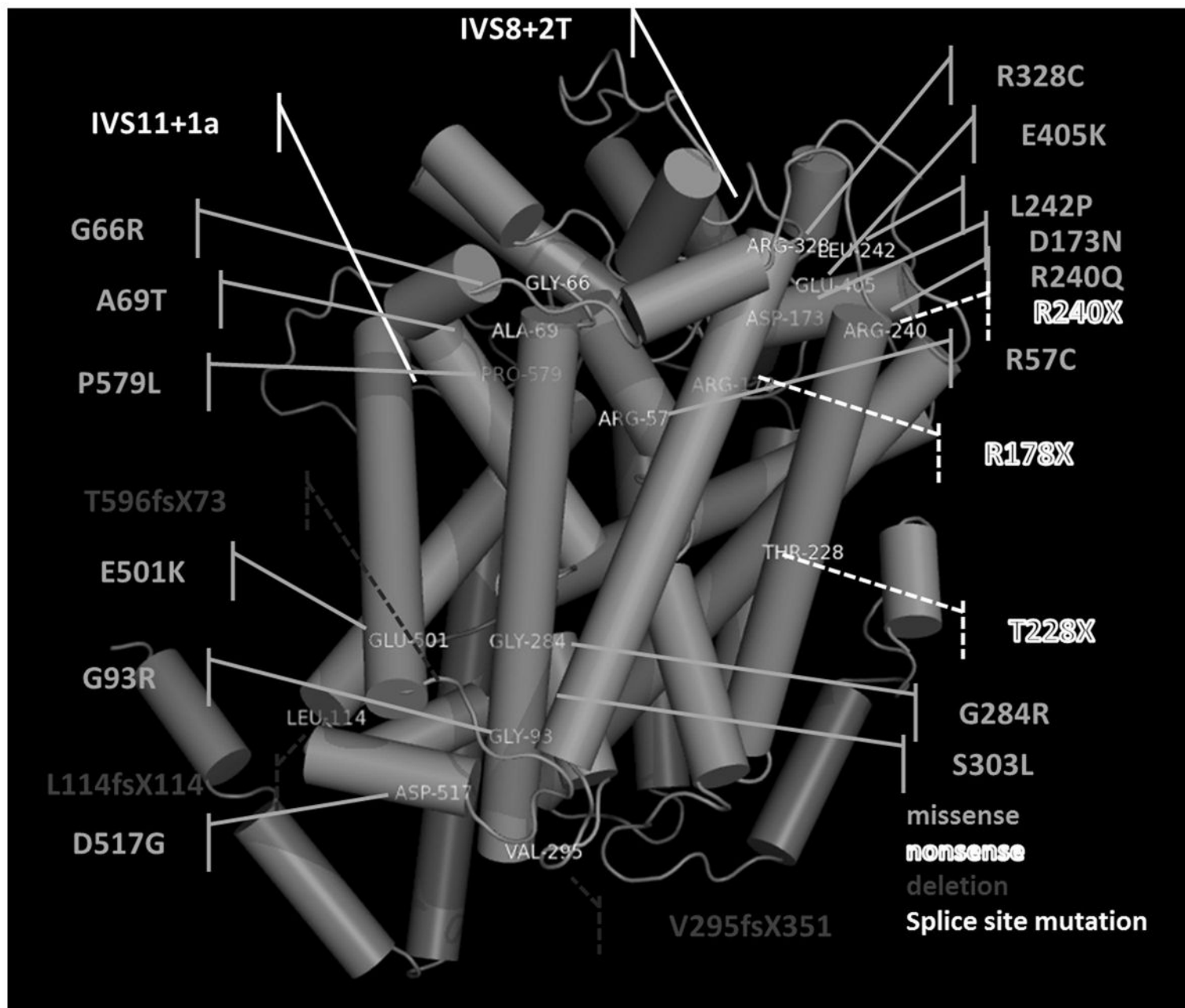
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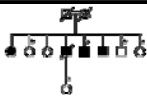



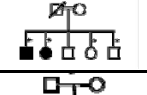














**Table 1: SLC6 human family members\*.**

Gene	Protein name	Key substrate	Ion dependence	Tissue distribution	chromosome
<b>SLC6A1</b>	GAT1	GABA	Na <sup>+</sup> , Cl <sup>-</sup>	central and peripheral neurons (gabaergic nerons)	3p25-p24
<b>SLC6A2</b>	NET , NAT1, NET1	norepinephrine	Na <sup>+</sup> , Cl <sup>-</sup>	central and peripheral neurons, adrenal gland, placenta	16q12.2
<b>SLC6A3</b>	DAT1 , DAT	dopamine	Na <sup>+</sup> , Cl <sup>-</sup>	brain (dopaminergic neurons)	5p15.3
<b>SLC6A4</b>	SERT , 5-HTT	serotonin	Na <sup>+</sup> , Cl <sup>-</sup> , K <sup>+</sup>	central and peripheral nervous system, epithelial cells, platelets	17q11.1-q12
<b>SLC6A5</b>	GlyT2	glycine	Na <sup>+</sup> , Cl <sup>-</sup>	spinal cord, brain (axon and presynaptic terminal of glycinergic neurons)	11p15.2-15.1
<b>SLC6A6</b>	TauT	taurine	Na <sup>+</sup> , Cl <sup>-</sup>	brain, retina, liver, kidney, heart, spleen, pancreas	3p25-p24
<b>SLC6A7</b>	PROT	L-proline	Na <sup>+</sup> , Cl <sup>-</sup>	brain (glutamatergic neurons)	5q31-q32
<b>SLC6A8</b>	CT1, CRTR	creatine	Na <sup>+</sup> , Cl <sup>-</sup>	ubiquitous	Xq28
<b>SLC6A9</b>	GlyT1	glycine	Na <sup>+</sup> , Cl <sup>-</sup>	brain, pancreas, uterus, stomach, spleen, liver, lung	1p33
<b>SLC6A11</b>	GAT3, GAT-B, GAT-4	GABA	Na <sup>+</sup> , Cl <sup>-</sup>	brain (gabaergic neurons, glia)	3p25.3
<b>SLC6A12</b>	BGT1	betaine, GABA	Na <sup>+</sup> , Cl <sup>-</sup>	kidney, brain	12p13
<b>SLC6A13</b>	GAT2, GAT3	GABA	Na <sup>+</sup> , Cl <sup>-</sup>	brain (meninges, ependymal, choroid plexus), retina, liver, kidney	12p13.3
<b>SLC6A14</b>	ATB <sup>0,+</sup>	neutral, cationic amino acids	Na <sup>+</sup> , Cl <sup>-</sup>	lung, trachea, salivary gland, mammary gland, stomach, colon, pituitary gland	Xq23-q24
<b>SLC6A15</b>	B <sup>0</sup> AT2, v7-3, NTT7-3	L-isoleucine	Na <sup>+</sup>	brain (amygdala, putamen, corpus callosum)	12q21.3
<b>SLC6A16</b>	NTT5	Orphan transport		testis, pancreas, prostate	19q13.1 - q13.4
<b>SLC6A17</b>	NTT4, XT1	neutral amino acids	Na <sup>+</sup>	synaptic vesicles	1p13.3
<b>SLC6A18</b>	XT2, B <sup>0</sup> AT3	Glycine, L-glutamine	Na <sup>+</sup> , Cl <sup>-</sup>	kidney (apical)	5p15.33
<b>SLC6A19</b>	B <sup>0</sup> AT1, XT2s1	neutral amino acids	Na <sup>+</sup>	intestine and renal proximal tubule (apical)	5p15.33
<b>SLC6A20</b>	SIT1, XT3s1, Xtrp3	imino acids	Na <sup>+</sup> , Cl <sup>-</sup>	intestine, kidney proximal tubule, choroid plexus, microglia, meninges of the brain, ovary	3p21.3

\*This table is based on the references cited along the section describing the amino acid transporters. For the monoamine, GABA and osmolyte transporter based on the work of Chen, Reith and Quick.<sup>2</sup> The hypothetical proteins encoded by the genes *SLC6A10* and *21* were not included in this table.

**Table 2: Hartnup causing mutations – Identified mutations in the *SLC6A19* gene encoding B<sup>0</sup>AT1.**

Pedigree	Family	Mutation	Zygosity	Symptoms
	Hartnup family UK <i>a</i>	IVS8+2T→G	homozygous	Aminoaciduria, mild cerebral ataxia, light sensitive skin rash (pellagra-like)
	Japan <i>a</i>	884-885del TG (V295fsX351)	homozygous	I) Mental retardation, gait disturbances, sunlight skin rash II) Psychological symptoms, ataxia and diplopia.
	Japan <i>a</i>	169C→T (R57C)	homozygous	Aminoaciduria, low plasma amino acid levels, light sensitive rash (pellagra-like)
	Japan <i>a</i>	340delC (L114FSX114) 682-683AC→TA (T228X)	Compound heterozygous	Aminoaciduria, asymptomatic
	USA <i>a</i>	517G→A (D173N)	Homozygous	Aminoaciduria, asymptomatic
	Australia <i>b</i>	IVS8+2G 725T→C (L242P)	Compound heterozygous	Aminoaciduria, asymptomatic
	Australia	517G→A (D173N) 1501G→A (E501K)	Compound heterozygous	Aminoaciduria, asymptomatic
	Australia <i>b, f</i>	IVS11+1A 719G→A (R240Q)	Compound heterozygous	Aminoaciduria, asymptomatic
	Australia <i>b</i>	517G→A (D173N)	homozygous	Aminoaciduria, asymptomatic
	Australia <i>b, f</i>	517G→A (D173N) 196G→A (G66R)	Compound heterozygous	Aminoaciduria, asymptomatic
	Australia <i>b, f</i>	517G→A (D173N) 1550A→G (D517G)	Compound heterozygous	Aminoaciduria, asymptomatic
	Australia <i>b</i>	517G→A (D173N) 718C→T (R240X)	Compound heterozygous	Aminoaciduria, asymptomatic
	Denmark <i>c</i>	725T→C (L242P) 794C→T (P265L)	Compound heterozygous	Pellagra-like skin rash, atactic gait
	Denmark <i>c</i>	IVS8+2T→G 205C→A (A69T)	Compound heterozygous	Aminoaciduria, asymptomatic
	Denmark <i>c</i>	IVS8+2T→G 1735C→G (P579L)	Compound heterozygous	Aminoaciduria, asymptomatic
	Germany <i>c</i>	277G→A (G93R)	homozygous	Aminoaciduria, asymptomatic
	China <i>d</i>	850G→A (G284R)	homozygous	Edema, involuntary movements of head and bilateral upper extremities history of dermatitis caused by photosensitivity.



	Korea <i>e</i>	c.908C>T (S303L) c.1787_1788insG (T596fsX73)	Compound heterozygous	seizures, attention-deficit hyperactivity disorder, and mental retardation without pellagra or ataxia, aminoaciduria
	Australia <i>f</i>	196G→A (G66R)/277G→A (G93R) 277G→A (G93R)/517G→A (D173N) 517G→A (D173N)/850G→A (G284R) 517G→A (D173N)/1550A→G (D517G)	Compound heterozygous  Compound heterozygous  Compound heterozygous  Compound heterozygous	Aminoaciduria, asymptomatic
	Canada <i>f</i>	532C→T (R178X)/1213G→A (E405K) 517G→A (D173N)/982C→T (R328C)	Compound heterozygous  Compound heterozygous	Aminoaciduria, asymptomatic Newborn screening

*a*,<sup>90,100</sup>; *b*,<sup>93,101</sup>; *c*,<sup>11</sup>; *d*,<sup>92</sup>; *e*,<sup>91</sup>; *f*,<sup>102</sup>.

**Table 3: SLC6A19 missense mutations shown to interact with Tmem27 and/or ACE2.** From the mutations co-expressed with the two accessory proteins, the majority caused a loss of surface expression (R57C, G93R, L242P, E501K, P579L) and the transporters were not functional alone or in co-expression. Two mutations had low function as shown for the wild type expressed alone, but prevented both accessory proteins of stimulating the transport function (A69T and R240Q). Two mutations led to a differential interaction with the accessory proteins (D173N and P265L), only the intestinal accessory protein triggers an increase in cell surface expression and function.<sup>9,</sup>  
<sup>11</sup> Several mutations were not yet assayed with the accessory proteins.

Protein	Position in the protein	Co-expression with	Membrane expression	Function
R57C	1st TMS	ACE2 and Tmem27	-	-
A69T	2nd TMS	ACE2 and Tmem27	↑	=
G93R	2nd TMS	ACE2 and Tmem27	-	-
D173N	2nd EL	ACE2	↑	↑
R240Q	3th EL	ACE2 and Tmem27	=	=
L242P	3th EL	ACE2 and Tmem27	-	-
P265L	6th TMS	ACE2	↑	↑
E501K	10th TMS	ACE2 and Tmem27	-	-
P579L	6th TMS	ACE2 and Tmem27	-	-
G66R	2nd TMS	nd		
G284R	6th TMS	nd		
S303L	7th TMS	nd		
R328C	7th TMS	nd		
E405K	4th EL	nd		
D517G	5th IL	nd		

nd, the function or membrane expression of these missense mutant were not yet determined in the presence of ACE2 or Tmem27